ACQUISITION AND LOSS OF POTENTIAL FOR MOTILITY OF SPERMATOZOA OF THE JAPANESE EEL *ANGUILLA JAPONICA*

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ABSTRACT

To date, cultivated male Japanese eels have not matured sexually in captivity under normal conditions. However, spermatogenesis and spermiation can be induced by the injection of gonadotropins. During this study, most males spermiated after the fifth or sixth weekly injection of human chorionic gonadotropin (HCG; 1 IU/g BW/wk) and the milt weight gradually increased as the number of injections increased. Motility of spermatozoa (percent motility after dilution with 450 mM NaCl) from HCG injected males showed significant individual differences and periodical changes after each weekly injection of HCG. Motility of spermatozoa in Japanese eel milt was found to have an intimate relationship between the pH of the milt and concentration of potassium in the seminal plasma. Motility of spermatozoa could be regulated by changes in the ionic constituents of the isotonic incubation media before dilution with a hyperosmotic solution. The percent motility of spermatozoa in eel milt increased significantly after incubating for 60 min in isotonic artificial seminal plasma (ASP), which consists of NaCl + KCl + $CaCl_2$ + $MgCl_2$ + $NaHCO_3$ buffered with TAPS-NaOH at pH 8.1, and with Ca^{2+}, Mg^{2+} free-ASP. Motility, however, decreased rapidly in K^+ free-ASP and in HCO_3^- free-ASP. These results indicate that acquisition and loss of the potential for motility of eel spermatozoa can be altered by changing the potassium and bicarbonate ion concentrations of the incubating medium irrespective of the initial potential for motility. The methods to obtain good quality spermatozoa described in this experiment should compensate for low volume of milt at the time of artificial fertilization.

INTRODUCTION

The freshwater Japanese eel *(unagi)* is one of the most widely cultivated species in Japan due to its popularity as a food fish. This popularity has caused a decrease in wild fry over the last 25 yr, as the eel fry under cultivation are wild-caught elver which have been captured in estuaries. The shortage of fry for cultivation has recently become a serious problem, leading to the intensive study of techniques for artificial breeding of the eel. In the present study, we investigated the factors which affect the milt quality in males artificially induced to mature, and examined the techniques for controlling the motility of eel spermatozoa.

INDUCTION OF TESTICULAR MATURATION BY HCG INJECTION

Cultivated male Japanese eels at 200-300 g BW are sexually immature and do not mature under normal culture conditions (Yamamoto et al. 1972). However, injection of gonadotropins can easily induce spermatogenesis and spermiation in the male for purposes of artificially propagating this species (Yamamoto et al. 1972). Chiba et al. (1997) reported that injections of human chorionic gonadotropin (HCG) administered to male eels less than 29 cm BL had no effect on the serum 11-ketotestosterone (11KT) levels, a major androgen in the eel (Miura et al. 1991), or on spermatogenesis. However, in fish more than 32 cm BL, testicular maturation was induced, accompanied by increases in plasma 11-KT levels. HCG has been used exclusively for the induction of sexual maturation in male Japanese eels, and a single injection of HCG at a relatively high dose (5 IU/g BW, Miura et al. 1991; 8 IU/g BW, Ohta and Tanaka 1997) is reportedly effective in inducing spermatogenesis.

To develop techniques for the artificial maturation of male eels, we investigated the number of weekly HCG injections required to obtain an adequate volume of high quality milt (Ohta et al. 1996a). Ten sexually immature males (252 g average BW) received 14 weekly injections of HCG (250 IU/eel/wk). Two out of 10 males spermiated after the fifth injection, and most had

spermiated after the sixth. The expressible milt weight gradually increased as the number of injections increased and became stable after injections 11-13. Percent motility of the spermatozoa measured after dilution with 450 mM NaCl increased after 7-9 injections and reached about 60-70% after the tenth injection. These results indicated that repeated weekly injections of HCG at 1 IU/g BW over 10 wk artificially induced sexual maturation in immature male eels.

PROBLEMS WITH INDUCTION OF TESTICULAR MATURATION

Although artificial induction of maturation in the immature male eel is not difficult, the milt obtained from an artificially matured male has not been sufficient in quantity or quality for high fertilization of eggs. First, the amount of obtainable milt from one male is small compared to the egg volume from one female. As already described, milt can be obtained from most of the males which received 10 or more weekly injections of HCG. The milt volume averages about 1 g (Ohta et al. 1996a), while the egg weight from one ovulated female often exceeds 300 g. Therefore, techniques must be developed to enable successful fertilization using the small quantity of milt.

Another problem is that individual differences in sperm motility are significant among the males induced to mature by artificial means. For example, Fig. 1 shows the percent motility of the milt from 10 randomly selected males which received 14 injections of HCG. Although the mean motility value was 55.3±8.2%, the lowest value was less than 10% and the highest more than 90%. It is clear that these differences in milt quality among males will lead to varying degrees of success in the fertilization of eggs. Furthermore, sperm motility changes with time after the administration of HCG. The changes in percent motility of spermatozoa in milt during the weekly injections of HCG are presented in Fig.2. The motility increased sharply 6 h after the injection and peaked after 24 h. Then, motility decreased by d 3 after injection, and remained at low level when tested on d 7. Milt obtained just prior to injection13 also showed low sperm

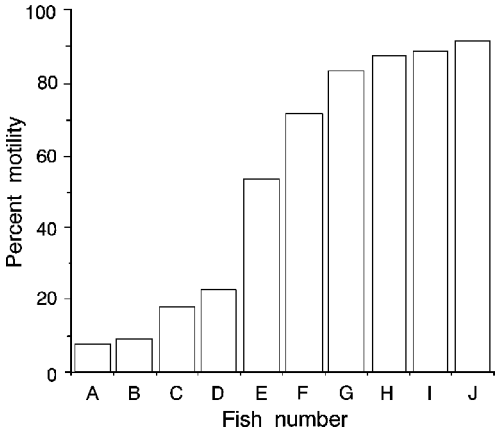

Figure 1. Percentage of motile spermatozoa of the milt from ten randomly selected males when the milt was diluted at 1,000 times with 450 mM NaCl buffered with 20 mM HEPES-NaOH at pH 7.5. Spermatozoa were classed as motile when the sperm head showed forward movement at 15 sec after dilution when analyzed under the VTR-light microscope.

motility. These periodic changes in percent motility will certainly affect the fertilization of eggs if the qualitative changes in milt quality at the time of artificial fertilization are disregarded. One possible solution to this problem is to give an additional injection of HCG 1 d prior to artificial fertilization (Ohta et al. 1997c).

RELATIONSHIP BETWEEN IONIC CHARACTERISTICS OF SEMINAL PLASMA AND SPERM MOTILITY

Changes in sperm motility observed during the weekly administration of HCG suggests

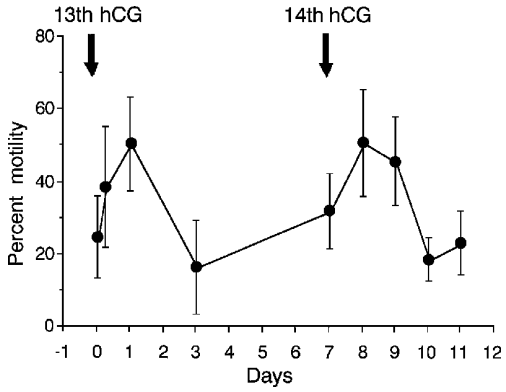

Figure 2. Changes in sperm motility during weekly injections of HCG (1 IU/g BW/wk). A small amount of milt was obtained from each male (n=5) just prior to injection 13, and 6 h, 24 h, and 72 h after, and just prior to injection 14, and 24 h, 48 h, 72 h, and 96 h after.

that the increase or decrease of HCG concentration affects the aqueous environment surrounding the spermatozoa in the sperm duct. Therefore, the potential for changes in the motility of spermatozoa appear to be related to the time elapsed after administration of HCG. It is possible that the composition of the seminal plasma can also affect the motility of the eel spermatozoa.

The biochemical characteristics of seminal plasma in 109 male Japanese eels were investigated with reference to sperm motility. After measuring the pH of the milt, the percent motility of spermatozoa and the ionic concentration of the seminal plasma in the milt were measured, respectively. An intimate relationship was found between sperm motility, milt pH and potassium concentration in the seminal plasma. The mean milt pH was 8.05 ± 0.02 and the potassium concentration of the seminal plasma was 20.85 ± 0.52 mM (n=109). We classified the milt from the 109 males into four groups: 1) milt which showed both milt pH and potassium concentration of seminal plasma more than the mean values (n=29), 2) milt which showed milt pH more than the mean value and potassium concentration less than the mean value (n=12), 3) milt which showed milt pH less than the mean value and potassium concentration more than mean value (n=24), and 4) milt which had both pH and potassium concentration less than the mean values (n=44). The mean percent motility of spermatozoa in the milt of these four groups is shown in Fig. 3. Percent motility of spermatozoa in the milt, of which pH and potassium concentration of seminal plasma were above the means, were significantly (P<0.05) higher than samples with both values less than the means. Similar relationships could not be found for other cations (Na^+, Ca^{2+}, or Mg^{2+}). These results indicate that the increase of potassium ions and decrease of protons in the seminal plasma stimulate the acquisition of sperm motility in the Japanese eel.

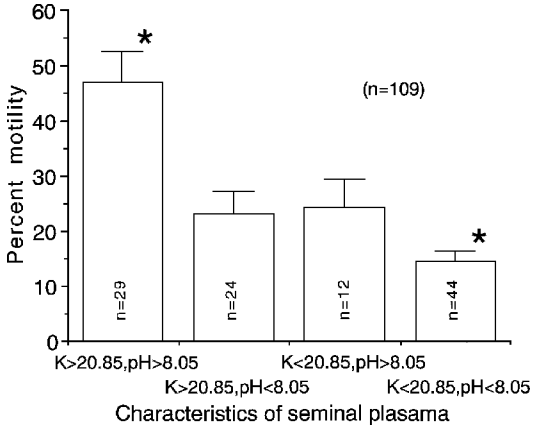

Figure 3. Relationship between percent motility of the spermatozoa when diluted with 450 mM NaCl and pH of the milt, and concentration of potassium ions in the seminal plasma. Asterisks mean significant difference (P<0.05) between them.

ACQUISITION AND LOSS OF POTENTIAL FOR MOTILITY IN THE MILT SPERMATOZOA *IN VITRO*

An artificial seminal plasma consisting of 149.3 mM NaCl + 15.2 mM KCl + 1.3 mM $CaCl_2$ + 1.6 mM $MgCl_2$ + 20 mM $NaHCO_3$ (buffered with 20 mM TAPS-NaOH at pH 8.1) was produced during the current study based on previous measurements of seminal plasma (Ohta et al. 1997a). Spermatozoa in milt was incubated in the ASP and changes in the potential for motility following dilution with 450 mM NaCl were recorded (Fig. 4). Before incubation, spermatozoa in the milt was observed at 53.4 ± 11.8% motility (initial control). The motility increased significantly when the milt was incubated with the ASP or Ca^{2+} and Mg^{2+} free ASP for 60 min (83.4 ± 2.5% and 86.1 ± 2.1%, respectively). In contrast, spermatozoa incubated with K^+ free ASP or HCO_3^- free ASP showed a sharp decrease in motility within 30 min, and reached 1.8 ± 0.7 %

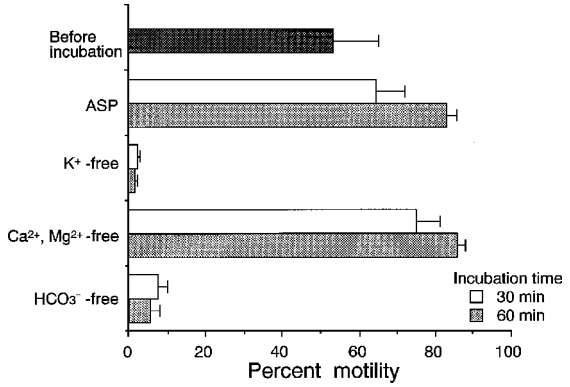

Figure 4. Effects of cations or anions in the ASP on the acquisition and loss of sperm motility. Horizontal bars indicate means ± SEM of results from 10 eels.

or 5.7 ± 2.2%, respectively. These results indicate that potassium and bicarbonate ions are essential for the acquisition and maintenance of motility of eel spermatozoa.

SOLUTIONS TO TECHNICAL PROBLEMS

The main problems with artificial induction of testicular maturation in the Japanese eel are: 1) the scarcity of milt compared with egg volume for successful fertilization, 2) the extent of individual differences in sperm motility among males artificially induced to mature, and 3) the change in motility with time after injections of HCG.

Low milt volume can be resolved by dilution of milt with appropriate diluent(s). Milt dilution has been reported to improve percent fertilization compared to undiluted milt which is low in volume (Poon and Johnson 1970; Billard et al. 1974; Rieniets and Millard 1987). Billard (1992) stressed the advantages of using diluents for artificial insemination compared to the dry method, and indicated the optimum sperm dilution of milt in salmonids is around 1000 times. In earlier studies of the Japanese eel, we reported the validity using artificial seminal plasma to dilute the milt up to 30 times (Ohta et al. 1997b) and 100 times (Ohta et al. 1996b; Kagawa et al. 1998) to improve percent fertilization.

Results from the present study suggests that the cause of the other two problems (individual differences and changes over time in sperm motility) is the lack of an effective regulatory mechanism of potassium and bicarbonate ions and/or proton concentrations in the seminal plasma of males treated with HCG. Although spermatogenesis can be readily induced by multiple injections of HCG, further improvement in hormonal treatment appears to be necessary for obtaining high quality milt.

A relationship between motility and the capacity for fertilization of teleost spermatozoa has been confirmed by several authors (Billard and Cosson 1992; Harvey and Kelley 1984; Ohta et al. 1995). The present study indicates that the potential for sperm motility in the Japanese eel can be regulated by incubating milt in an isotonic incubation media with a high concentration of potassium and bicarbonate ions. Techniques for obtaining good quality spermatozoa of the Japanese eel by the incubation method used in the current investigation should compensate for the shortage of milt at the time of artificial fertilization and improve percent fertilization.

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